Analysis of *maxillopedia* Expression Pattern and Larval Cuticular Phenotype in Wild-Type and Mutant Tribolium

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Manuscript received December 13, 1999
Accepted for publication February 22, 2000

ABSTRACT

The *Tribolium castaneum* homeotic gene *maxillopedia* (*mxp*) is the ortholog of Drosophila *proboscipedia* (*pb*). Here we describe and classify available *mxp* alleles. Larvae lacking all *mxp* function die soon after hatching, exhibiting strong transformations of maxillary and labial palps to legs. Hypomorphic *mxp* alleles produce less severe transformations to leg. RNA interference with *maxillopedia* double-stranded RNA results in phenocopies of *mxp* mutant phenotypes ranging from partial to complete transformations. A number of gain-of-function (GOF) *mxp* alleles have been isolated based on transformations of adult antennae and/or legs toward palps. Finally, we have characterized the *mxp* expression pattern in wild-type and mutant embryos. In normal embryos, *mxp* is expressed in the maxillary and labial segments, whereas ectopic expression is observed in some GOF variants. Although *mxp* and Pb display very similar expression patterns, *pb* null embryos develop normally. The *mxp* mutant larval phenotype in Tribolium is consistent with the hypothesis that an ancestral *pb*-like gene had an embryonic function that was lost in the lineage leading to Drosophila.

THE homeotic selector genes of the fruit fly, *Drosophila melanogaster*, assign developmental fate to cells appropriate to their location along the anterior-posterior axis (Denell *et al.* 1996). Mutations in these genes often produce transformations of one body region to another. Homeotic genes are very ancient; a single cluster arose very near the origin of the Eumetazoa (Finnerty 1998). In *D. melanogaster*, this cluster has been split into two complexes: the Antennapedia complex (ANTC; Kaufman *et al.* 1990) and the bithorax complex (BXC; Lewis 1978). In recent years homeotic genes have been cloned from a number of organisms to study how changes in expression and/or targets of homeotic genes may have played an important role in the morphological evolution of animals (Averof and Patel 1997; Rogers *et al.* 1997).

Drosophila and other higher flies are specialized with respect to embryonic cephalic development. The head and gnathocephalic segments involute through the presumptive mouth and contribute predominately to internal larval structures. The effects of homeotic mutations on the larval head differ dramatically from the transformations produced in adult heads. This observation led Diederich *et al.* (1991) to argue that the embryonic functions of the ANTC genes affecting these segments have been modified from those typical of most insects, and that in fact the adult mutant abnormalities associated with these genes are a better guide to their ancestral regulatory roles.

The ANTC gene *proboscipedia* (*pb*) is a particularly striking example of the dichotomy between adult and embryonic homeotic mutant phenotypes. *pb* is unique among Drosophila homeotic genes in that it is completely dispensable for normal embryonic development (Pultz *et al.* 1988). This lack of embryonic function is somewhat surprising since an ancestral *pb* gene existed as part of the Homeotic complex before the separation of protostomes and deuterostomes (Ruddle *et al.* 1994) and presumably shared with its homeotic neighbors a role in assigning developmental fate during embryogenesis. In contrast, adult flies lacking *pb* gene function exhibit dramatic transformation of the proboscis, or labium, to prothoracic legs, as well as a reduction in size of the maxillary palps that may represent a transformation toward antennae (Bridges and Dobzhansky 1933; Kaufman 1978).

Studying the function of a *pb* ortholog in a less derived insect could provide valuable insight into whether an ancestral *pb* gene had an embryonic function, and if so what that function may have been. The red flour beetle, *Tribolium castaneum*, exhibits a mode of embryogenesis more typical of insects and is currently the only non-Drosophilid insect for which full-scale genetic studies are feasible. In work published elsewhere, we have cloned the Tribolium ortholog of *pb* and have demonstrated that it corresponds to the genetically ascertained *maxillopedia* locus (Shippy *et al.* 2000).

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maxillopedia (*mxp*) was first defined by the spontaneous, recessive-viable mutation mxp^{1} (formerly *mxp*), which weakly transforms maxillary and labial appendages toward legs in both adults and larvae (Hoy 1966). Beeman *et al.* (1989) identified a group of additional *mxp* mutations that are believed to include nulls, hypomorphs, and gain-of-function (GOF) alleles. The putative null *mxp* alleles cause strong transformation of larval maxillary and labial palps to legs and lethality about the time of the first larval molt. Thus, unlike *pb*, *mxp* function is required for proper identity of larval as well as adult mouthparts. It is also striking that GOF mutations of *mxp*, most of which produce dominant transformations of the legs and/or antennae to palps, are quite common, whereas only one *pb* GOF mutation has been described (Cribbs *et al.* 1992).

Here we classify available *mxp* alleles based on genetic analysis and larval mutant phenotype. Furthermore we demonstrate that both null and hypomorphic *mxp* alleles are phenocopied by RNA interference. We describe the expression pattern of *mxp* transcripts in wild-type embryos and show that it closely resembles the pattern described for Pb protein (Pultz *et al.* 1988). In addition, we demonstrate that several GOF *mxp* mutations are associated with ectopic *mxp* expression and, in some cases, loss of expression in all or part of the normal domain.

MATERIALS AND METHODS

Beetle strains and genetic analysis: Beetle cultures were maintained at 30° as described by Beeman *et al.* (1989). For egg collection, beetles were transferred to Gold Medal flour (General Mills) supplemented with 5% brewer's yeast. Strains used were mxp^{170}/A^{Es}; mxp^{19}/A^{Es}; Lu^{R1}/Ey; mxp^{1}, *apt*, pas^{30}/mxp^{1}, *apt*, pas^{30}; mxp^{8}/A^{Es}; mxp^{lp}/A^{Es}; mxp^{X9}, A^{Es}/Hw; mxp^{Ng}/A^{Es}; mxp^{Stbd}/A^{Es}; mxp^{Stb}/Ag; $mxp^{Dch\text{-}1}/A^{Es}$; $mxp^{Dch\text{-}4}/A^{Es}$; $mxp^{Dch\text{-}3}/Ey$; mxp^{Stm}/mxp^{Stm}; mxp^{Stm}/A^{83} and mxp^{StmR1}/A^{Es}. For analysis of gene expression, strains were first outcrossed to wild type and then either self-crossed or outcrossed a second time. Novel expression patterns observed only in self-cross progeny were interpreted as representing the homozygous mutant class.

Documentation of larval phenotypes: Eggs were collected from balanced stocks or crosses of *mxp* mutants and then incubated at 30° until hatching. Hatched larvae were transferred into 9:1 lactic acid:ethanol and heated at 60° for 1–3 days to clear. Larvae to be photographed were mounted under a coverslip in PVLP mounting medium (8.4% polyvinyl acetate, 22% lactic acid, 22% phenol). Nomarski images from an Olympus BX50 microscope were captured with a Kontron Progres 3012 digital camera. Contrast, brightness, and sharpness were adjusted using Adobe Photoshop software.

RNA-mediated interference: Double-stranded RNA (dsRNA) was synthesized as described by Brown *et al.* (1999) except that transcription was performed using MEGAscript SP6 and T7 polymerase kits (Ambion) according to the manufacturer's instructions. Two *mxp* templates were used for RNA synthesis: a nonhomeobox-containing 903-bp subclone from the 3′ end of the *mxp* transcript, and a 1.5-kb partial cDNA that includes the homeobox (Shippy *et al.* 2000). dsRNA were diluted with injection buffer (5 mm KCl, 0.1 mm KPO_4, pH 6.8, and green food dye) and microinjected into 0–4-hr Tribolium embryos as described by Brown *et al.* (1999). The nonhomeobox dsRNA (8.5 μg/μl) was diluted to 7.7 μg/μl, while the homeobox dsRNA (1.87 μg/μl) was diluted to 0.93 μg/μl. Injected embryos were incubated at room temperature in a humidified, oxygenated chamber. Approximately 1 week after injection, hatched larvae were treated with lactic acid and ethanol (9:1) and examined for abnormalities in cuticle morphology.

Analysis of gene expression: *In situ* hybridization was performed as described by Brown *et al.* (1994b). A digoxygenin-labeled antisense riboprobe was transcribed from a 3.8-kb homeobox-containing *Xho*I-*Hin*dIII fragment of *mxp* genomic DNA (Shippy *et al.* 2000 and unpublished results). Engrailed protein was detected with the crossreacting Mab4D9 antibody (Patel *et al.* 1989).

RESULTS

Analysis of *mxp* mutant alleles: We have used genetic analysis and larval cuticular phenotype to classify existing and newly identified *mxp* mutant alleles (see Table 1 for summary). The alleles fall into four categories: null alleles, hypomorphic alleles, those in which gain-of-function effects accompany loss of normal function (GOF/LOF), and those with normal function accompanied by GOF effects.

Null alleles: Several radiation-induced alleles of *mxp* identified by Beeman *et al.* (1989) cause recessive larval lethality with strong transformation of the maxillary and labial palps to legs and have been proposed to be null alleles. We have analyzed two putative null alleles: mxp^{170} and mxp^{19}. To determine whether these alleles truly represent *mxp* nulls, we utilized *Lucifer Revertant 1* (Lu^{R1}), a deficiency which removes the *mxp* locus. This deficiency will be described in detail elsewhere, but for the purposes of this article it is important to note that it deletes genes on either side of *mxp*. Lu^{R1} fails to complement alleles of *TcDeformed* (*TcDfd*) and has been shown molecularly to lack *Tclabial* (our unpublished results). The phenotypes of mxp^{19} (Figure 1B) and mxp^{170} (data not shown) homozygotes and hemizygotes (data not shown) are identical, suggesting that mxp^{19} and mxp^{170} are null alleles. Furthermore, mxp^{19} is associated with a single base pair deletion within the *mxp* coding region that is predicted to result in a truncated, nonfunctional protein (Shippy *et al.* 2000). We will use the notation mxp^{-} to represent a null allele of *mxp*.

The maxillary appendages of Tribolium are composed of a basal coxopodite, a distal telopodite (palp), and an endite lobe that projects ventrally from the coxopodite (Figure 1A). Only the telopodites are transformed to legs in mxp^{-} mutants; the endites are unaffected (Figure 1B). The coxopodites of wild-type labial appendages are fused at the ventral midline and lack discernable endites. Except for their slightly smaller size, the labial telopodites closely resemble the maxillary telopodites (Figure 1A). In mxp^{-} mutants, the labial telopodites are completely transformed into legs, but the coxopodites remain fused (Figure 1B). The appearance of the transformed larval appendages in null ho-

TABLE 1

Classification of *maxillopedia* alleles

Allele name (*mxp**)	Origin	Allele type	Homozygous lethal stage	Larval phenotype		Adult phenotype
				(*mxp**/*mxp**) transformation toward leg	(*mxp**/*mxp*$^{-}$) transformation toward leg	(*mxp**/*mxp*$^{+}$) structure affected
1	Spontaneous	Partial LOF	None	Weak MAX and LAB	Mod. MAX and LAB	None
8	γ-Irradiation	Partial LOF	L1	Mod. MAX, weak LAB		None
labiopedia (lp)	γ-Irradiation	Partial LOF	L1	Strong LAB	Strong LAB, mod. MAX	None
X9	γ-Irradiation	Partial LOF	Early embryonic	N/A	Strong MAX and LAB	None
19	γ-Irradiation	Null	L1	Strong MAX and LAB	Strong MAX and LAB	None
170	EMS	Null	L1	Strong MAX and LAB	Strong MAX and LAB	None
StumpyR1 (*StmR1*)	γ-Irradiation	Null	L1	Strong MAX and LAB		None
Notched gena (*Ng*)	γ-Irradiation	GOF/LOF	L1	Strong MAX and LAB	Strong MAX and LAB	Notched head capsule
Dachs-1 (*Dch-1*)	γ-Irradiation	GOF/LOF	L1	Weak MAX		Short antennae and legs
Dachs-4 (*Dch-4*)	γ-Irradiation	GOF/LOF	L1	Weak MAX and LAB		Short antennae and legs
Stubby (*Stb*)	EMS	GOF/LOF		N/A	Mod. MAX, weak LAB	Short antennae and legs
Stuboid (*Stbd*)	γ-Irradiation	GOF/LOF	L1	Strong MAX, weak LAB	Strong MAX, weak LAB	Short antennae and legs
Dachs-3 (*Dch-3*)	γ-Irradiation	GOF/LOF	Late embryonic	N/A	Strong MAX, weak LAB	Short T1 leg
Stumpy (*Stm*)	EMS	GOF				Short antennae
{*Antennapalpus* (*Apl*)}	EMS	GOF	None			Short antennae
{*Spatulate* (*Spa*)}	γ-Irradiation	GOF				Short antennae

mxp^{-}, *mxp* null; MAX, maxillary palps; LAB, labial palps; Weak, transformation of only the distal tips of palps to leg; Mod., moderate transformation involving distal and proximal structures but not resulting in a complete leg; Strong, complete transformation of palp to leg; blanks, not examined; bracketed alleles are putative *mxp* alleles; N/A, results could not be obtained due to early lethality.

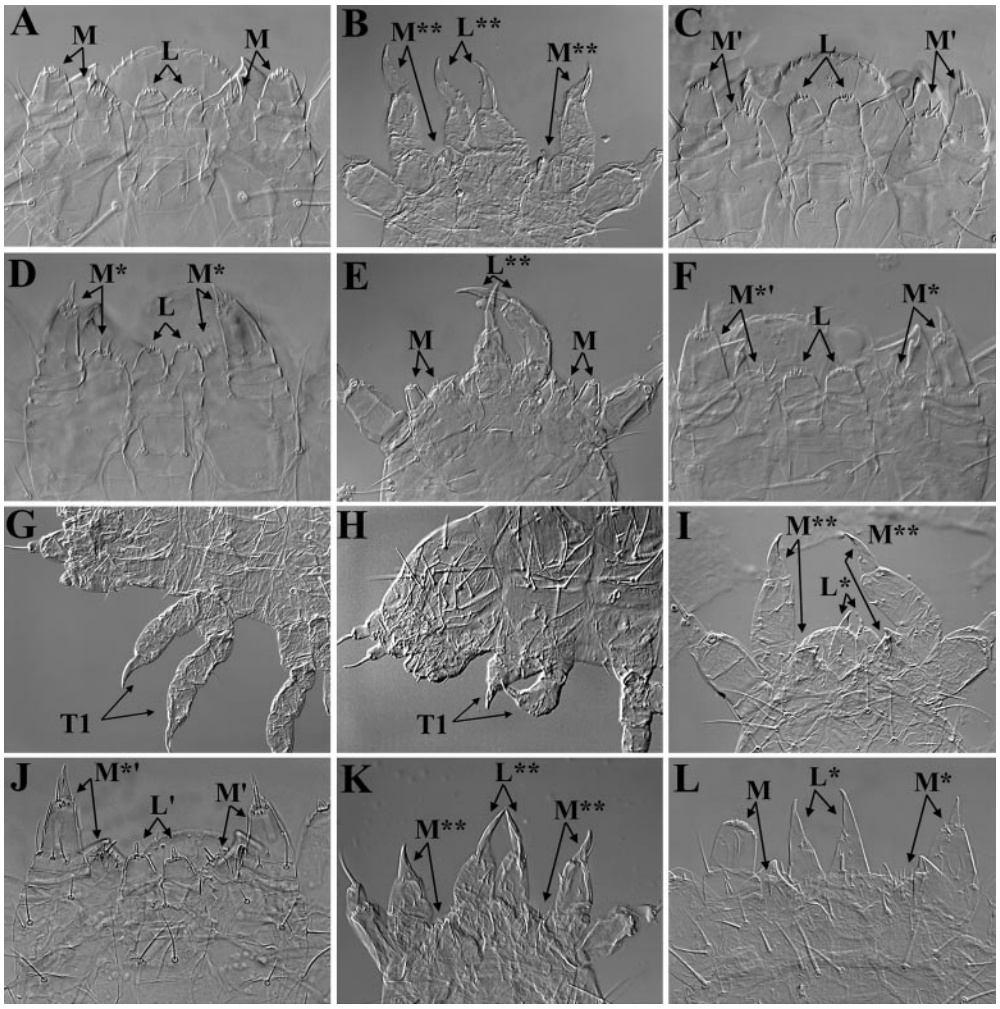

Figure 1.—Effect of *mxp* mutations on larvae. All images (except G and H) are ventral views with anterior at the top. (A) In the wild-type larval head each maxillary appendage (M) comprises a basal coxopodite with a ventral (medial) endite and a distal telopodite (palp). The labial appendages (L), which have no visible endites, are fused at the base and nest between the maxillary appendages. Numerous sensilla of various forms are found on the distal tips of the maxillary and labial palps. (B) A homozygous null (mxp^{19}/mxp^{19}) larva shows complete transformations of maxillary and labial palps to legs (M** and L**). (C) An $mxp^{19}/+$ larva appears morphologically normal except that an abnormally long spike is present on each maxillary palp (M′). (D) An mxp^{1}/mxp^{1} larva exhibits distal transformation of maxillary palps to leg (M*). In this individual, the labial palps appear normal. (E) The labial palps of an mxp^{lp}/mxp^{lp} larva are completely transformed to legs (L**). The maxillary palps occasionally show distal spikes or distal transformations to leg, but are normal in this individual. (F) An $mxp^{Dch\text{-}4}/mxp^{Dch\text{-}4}$ larva exhibits distal transformations of maxillary palps to leg(M*). In this individual, one maxillary palp also has a distal spike (M*′). The labial palps are sometimes distally transformed to legs, but in this individual they are normal. (G) A lateral view of a wild-type larva (anterior to the left) shows a normal T1 leg. (H) An $mxp^{Dch\text{-}3}/+$ larva has a much shorter than normal T1 leg. (I) An $mxp^{Dch\text{-}3}/mxp^{19}$ larva exhibits complete transformation of maxillary palps to leg (M**), but only distal transformation of labial palps to leg (L*). (J) The maxillary appendages of an $mxp^{Dch\text{-}1}/mxp^{Dch\text{-}1}$ larva are distally transformed to leg, but also have a distal spike (M*′) similar to the null heterozygote in C. The labial appendages also have a distal spike (L′). (K) Injection of *mxp* dsRNA into wild-type eggs results in transformations of the maxillary and labial palps similar to those produced by the null *mxp* allele shown in B. (L) Injection of lower concentrations of *mxp* dsRNA (approximately eightfold lower than in K) resulted in partial transformations of maxillary and labial palps to legs (M* and L*). One maxillary palp is normal in this individual.

mozygotes and hemizygotes is variable. While some of these individuals have morphologically normal legs (Figure 1, compare B to G) extending from the maxillary and labial segments, the transformed appendages of the remaining larvae are warped and shortened to varying degrees (not shown). Interestingly, the thoracic legs of these individuals (where *mxp* is not normally expressed) are also warped. Warped legs are sometimes observed in other (non-*mxp*) mutant strains, suggesting that this phenotype may be a nonspecific developmental abnormality.

To date, no haploinsufficient *mxp* phenotype has been reported. We have observed, however, that more than half of larvae heterozygous for a null *mxp* allele have an abnormally long spike on one or both maxillary palps (Figure 1, compare A and C). This phenotype appears to be an alteration of a normally paddle-shaped sensilla to a longer tapered structure.

Hypomorphic alleles: Beeman *et al.* (1989) interpreted the original *mxp* mutation, mxp^{1} (Hoy 1966), as a hypomorphic allele. Indeed, we have observed that mxp^{1}/mxp^{-} larvae and adults (data not shown) have more severe transformations than mxp^{1}/mxp^{1} individuals (Figure 1D). Several additional hypomorphic alleles have been induced by γ-irradiation. mxp^{8} homozygotes die at the first larval molt, but show incomplete transformations of mouthparts, suggesting that partial *mxp* function is retained. The terminal portions of the palps are longer than normal, and tarsal claws are often observed (data not shown). Occasionally, palps terminate in a

spike identical to that observed in null heterozygotes. *mxp*^{labiopedia} (*mxp*^{lp}; Beeman *et al.* 1989) is unique in that the labial palps of homozygous larvae are much more strongly transformed than the maxillary palps (which occasionally bear a spike or claw, but more often appear morphologically normal; Figure 1E). *mxp*^{lp} is apparently a hypormorphic allele since at least partial *mxp* function is present in the maxillary appendages. *mxp*^{X9} was identified in a mutagenesis of a chromosome carrying the *Abdominal* allele *Extra-sclerite* (*A*^{Es}; Beeman *et al.* 1989). The *A*^{Es} mutation suppresses recombination in the homeotic gene complex (HOMC), preventing separation of the two mutations, and is a recessive early embryonic lethal. Thus, the larval phenotype of *mxp*^{X9} homozygotes cannot be determined. To determine whether *mxp*^{X9} is associated with LOF at the *mxp* locus, we examined its phenotype when heterozygous with a null *mxp* allele. *mxp*^{X9}/*mxp*$^{-}$ heterozygotes die about the time of the first larval molt showing strong maxillary and labial transformations to leg similar to null alleles (data not shown). However, *mxp*^{X9}/*mxp*^{lp} heterozygotes are viable to adulthood and show only labial to leg transformations (data not shown). *mxp*$^{-}$/*mxp*^{lp} heterozygotes die as larvae (data not shown), indicating that *mxp*^{X9} is a hypomorphic allele.

GOF alleles that fail to complement the adult phenotype of mxp^{1}*:* Beeman *et al.* (1989) identified a number of *mxp* alleles that fail to complement *mxp*^{1} and, in addition, show dominant effects. These alleles were interpreted as having GOF as well as complete or partial LOF effects. One mutation, *mxp*$^{Notched\ gena}$ (*mxp*^{Ng}), produces notches in the anterior head capsule of the adult. Both *mxp*^{Ng} homozygotes and *mxp*^{Ng}/*Lu*^{R1} hemizygotes (data not shown) die as larvae and show complete maxillary and labial transformations to leg, suggesting that *mxp*^{Ng} has lost all normal *mxp* function. As observed for other *mxp* nulls, some *mxp*^{Ng} homozygotes show warping of both gnathal and thoracic legs. Apparently, *mxp*^{Ng} also has dosage-dependent larval GOF effects since homozygotes show larval head capsule abnormalities. However, no abnormal expression of *mxp* is observed in progeny of an *mxp*^{Ng}/*A*^{Es} self-cross (see below). If *mxp* is ectopically expressed in the larval head, it is at a level below that detectable in our assays.

Other dominant *mxp* mutations cause shortening of the antennae and/or legs in what is thought to be a transformation to palp (Beeman *et al.* 1989). Alleles in this class include *mxp*$^{Dachs-4}$ (*mxp*$^{Dch-4}$), *mxp*$^{Dch-3}$, *mxp-Stubby* (*mxp*^{Stb}, formerly *mxp*^{D2}), *mxp*$^{Dch-1}$, and *mxp-Stuboid* (*mxp*^{Stbd}, formerly *mxp*^{D3}).

The *mxp*$^{Dch-4}$ allele was recovered from a γ-ray mutagenesis screen (Beeman *et al.* 1989). *mxp*$^{Dch-4}$ homozygotes normally die as larvae, but Beeman *et al.* (1989) reported the discovery of an *mxp*$^{Dch-4}$ homozygous escaper that showed striking transformation of adult tibia and tarsi to palps, as well as shortened antennae. Heterozygous adults are less severely affected, but legs and antennae are noticeably shortened. No effect on larval legs or antennae has been observed. *mxp*$^{Dch-4}$ homozygous larvae show only mild LOF effects with variable expressivity (Figure 1, compare F to A). In the most severe cases, the distal tips of the maxillary and labial palps are transformed to tarsal claws. The maxillary appendages may also have the distal spike seen in *mxp* null heterozygotes. Some individuals have both a tarsal claw and a distal spike on the same maxillary palp, demonstrating that the spike is not simply an abnormal tarsal claw. Changes in the *mxp* expression pattern consistent with the *mxp*$^{Dch-4}$ phenotype have been noted in *mxp*$^{Dch-4}$ heterozygotes and homozygotes (see below).

mxp^{Dch-3}, a previously undescribed γ-ray-induced mutation, is unique in that it causes only the prothoracic (T1) legs to be shortened. This effect is seen in both adults (data not shown) and larvae (Figure 1, compare G and H). The *mxp*$^{Dch-3}$ mutation results in pseudolinkage of the HOMC with the LG9 locus *pearl* (R. W. Beeman, unpublished results), indicating that *mxp*$^{Dch-3}$ is associated with an LG2 to LG9 translocation. *mxp*$^{Dch-3}$ homozygotes die before cuticularization (presumably due to the LG9 breakpoint), but *mxp*$^{Dch-3}$/*mxp*$^{-}$ heterozygotes exhibit maxillary and to a lesser extent labial transformations to leg (Figure 1, compare I and A). These phenotypes are consistent with changes in the *mxp* expression pattern associated with the *mxp*$^{Dch-3}$ allele (see below).

mxp^{Stb} (Beeman *et al.* 1989) was recovered from an EMS mutagenesis and causes shortening of the antennae as well as slight shortening of the legs in adult heterozygotes. The larval antennae and legs do not appear to be affected. No homozygous class can be differentiated among progeny of an *mxp*^{Stb} heterozygous self-cross, suggesting that either these individuals die early in embryogenesis or *mxp*^{Stb} has no *mxp* recessive LOF phenotype. *mxp*^{Stb}/*mxp*$^{-}$ larvae, however, exhibit transformations of maxillary and to a lesser extent labial palps to legs (data not shown), indicating that *mxp*^{Stb} is associated with partial LOF. Among 531 progeny of an outcross of *mxp*^{Stb}/+ males, only one *mxp*^{Stb} male was recovered. However, both male and female *mxp*^{Stb} individuals are obtained from a balanced stock. Thus, *mxp*^{Stb} behaves as an X-linked gene and so is apparently associated with an LG2 to X translocation.

mxp^{Dch-1} (Sokoloff 1982) is a γ-ray-induced allele that causes pseudolinkage between LG2 and LG9 (Beeman *et al.* 1986). Homozygotes die about the time of the first larval molt and show moderate maxillary, but not labial, transformations to leg. The transformed maxillary appendages usually have both a distal spike (as seen in null heterozygotes) and a tarsal claw (Figure 1, compare J to A). The labial palps of *mxp*$^{Dch-1}$ homozygotes often have a distal spike as well. The legs and antennae of homozygous and heterozygous larvae appear normal, despite the presence of short legs and antennae in adult heterozygotes.

mxp^{Stbd} was induced by γ-irradiation (Beeman *et al.*

1989). Adult heterozygotes have shortened antennae and slightly shortened legs. mxp^{Stbd} homozygous larvae show moderate maxillary and weak labial transformations to leg (data not shown), but no apparent effect on larval antennae or legs.

GOF mutations that complement the adult phenotype of mxp^{1}*:* Three mutations that map to the HOMC and exhibit dominant antennal effects, *Antennapalpus* (*Apl*), *Spatulate* (*Spa*), and *Stumpy* (*Stm*), were predicted by Beeman *et al.* (1989) to be GOF *mxp* alleles. *Apl* and *Spa*, but not *Stm*, complement the larval lethality of *mxp* nulls. All three mutations were reported to complement the recessive adult phenotype of mxp^{1} (Beeman *et al.* 1989). Reversion experiments indicate that *Stm* is an *mxp* allele. Two events that completely reverted the dominant *Stm* phenotype were recovered among 31,500 irradiated chromosomes. Each reversion (*StmR1* and *StmR6*) fails to complement the adult phenotype of mxp^{1} and results in lethality when heterozygous with an *mxp* null. The reversions do not appear to affect multiple loci, since homozygous larvae display only the *mxp* null phenotype (data not shown). Thus, we will hereafter refer to *Stm* as mxp^{Stm}. mxp^{Stm} was identified in an EMS mutagenesis, but reduces the frequency of crossing-over in the vicinity of the HOMC, suggesting it may be a chromosomal rearrangement. We show below that mxp^{Stm} is associated with ectopic *mxp* expression, which is eliminated by reversion.

RNA interference: RNA-mediated interference (RNAi), a technique first used in the nematode *Caenorhabditis elegans*, has been adapted recently for use in Drosophila and Tribolium (Kennerdell and Carthew 1998; Brown *et al.* 1999; Misquitta and Paterson 1999). dsRNA corresponding to the 3′ end of the *mxp* cDNA was injected into wild-type Tribolium embryos. Of 103 hatching larvae, 100 exhibited transformations of the maxillary and labial palps to legs similar to those seen in *mxp* null larvae (Figure 1, compare B and K), indicating that *mxp* function had been severely reduced, if not eliminated. Injection of a lower concentration (approximately eightfold less) of an *mxp* dsRNA that included the homeobox produced less severe phenotypes, similar to those seen in *mxp* hypomorphs (Figure 1, compare L to D and I). The presence of the homeobox did not appear to result in crossreactivity with other homeobox-containing genes. The phenocopies of *mxp* mutant phenotypes produced by RNAi verify the conclusion of Brown *et al.* (1999) that RNAi in Tribolium should be useful for predicting the LOF phenotypes of genes for which no mutants exist.

***mxp* expression in wild-type embryos:** Expression of *mxp* was examined via *in situ* hybridization using a digoxygenin-labeled antisense riboprobe containing the *mxp* homeobox (Shippy *et al.* 2000). In most respects, the expression pattern is quite similar to that observed for Pb protein (Pultz *et al.* 1988). Neither Pb nor *mxp* is expressed before or during the blastoderm stage. In Drosophila, *pb* transcripts are detected on developmental Northerns at 2–4 hr, which corresponds with the stages of gastrulation and germband elongation. *mxp* expression in Tribolium also appears prior to morphological signs of segmentation, as the newly formed germ rudiment begins to elongate. Tribolium embryogenesis is quite different from that of Drosophila, in that the segments form one at a time rather than virtually simultaneously (Brown *et al.* 1994a). The visible formation of each segment is presaged by expression of the Tribolium Engrailed homolog (TcEn) in what will become the posterior compartment of that segment. Thus, TcEn serves as a useful indicator of embryonic stage, as well as providing a framework in which to interpret other expression patterns. *mxp* transcripts are first detected in a small group of mesodermal cells at the ventral midline of the mandibular segment in embryos with four to five Engrailed stripes (Figure 2A). As the embryo develops, expression in the mandibular segment broadens into two clusters (Figure 2B). By the time nine Engrailed stripes have formed, staining in the mandibular mesoderm is quite intense, and weak transient expression appears at the ventral midline of the maxillary segment (Figure 2C). As germband elongation continues, *mxp* expression intensifies in the mesoderm of the developing mandibular appendages. At this time, faint *mxp* expression is detected in the maxillary and labial limb buds (Figure 2D). By the time segmentation is complete, strong *mxp* expression is detected in both ectoderm and mesoderm of the maxillary and labial limb buds (Figure 2, E and F). Faint expression is still detected in the mesoderm of the mandibular limbs. As germband retraction begins, *mxp* expression is detected in two clusters of cells flanking the ventral midline of the intercalary segment (Figure 2G). These cells lie below the ventral surface of the embryo and are most likely part of the developing central nervous system (CNS). Staining in the maxillary and labial appendages intensifies still further as the labial palps assume their larval position nested between the bifurcated maxillary appendages (Figure 2H). *mxp* expression is visible in both the telopodites and endites of the maxillary appendages. At this stage, *mxp* is also expressed in a subset of cells in the ganglia of each segment from the maxillary segment through the posterior tip of the embryo.

The epidermal expression pattern of *mxp* is quite similar to that of Pb if the maxillary and labial lobes of Drosophila are considered equivalent to the maxillary and labial appendages of Tribolium embryos.

***mxp* expression in mutant embryos:** We have also analyzed embryonic *mxp* expression in a number of mutant strains (see Table 2 for summary of results). *mxp*/balancer heterozygotes were mated to wild-type beetles. Heterozygous progeny, recognizable at the adult stage by the absence of the marked balancer chromosome (and in some cases by the presence of an *mxp* allele-

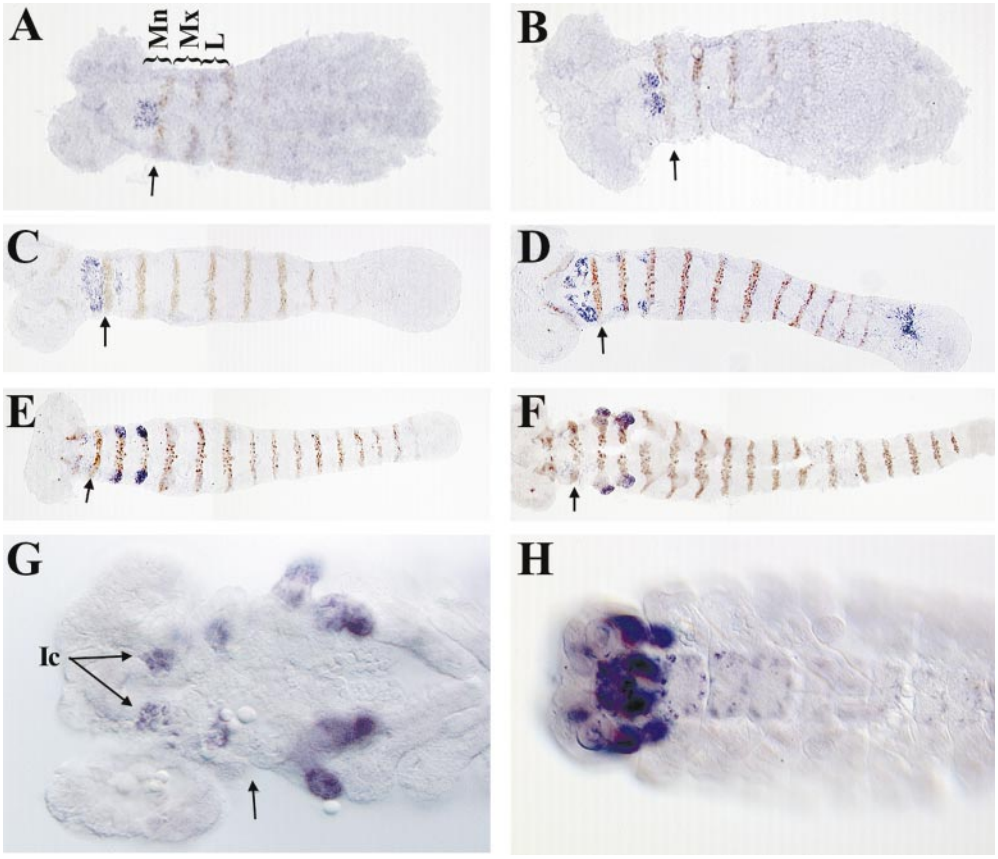

Figure 2.—Wild-type *mxp* expression pattern during embryogenesis. Embryos in A–F are double-stained with *mxp* riboprobe (purple) and a cross-reacting Engrailed (En) antibody (brown). The embryos in G and H were not stained with En antibody. An arrow points to the mandibular En stripe in A–F and its approximate position in G. All views are of the ventral surface of an embryo with anterior to the left. (A) *mxp* is first expressed in the mandibular mesoderm. The mandibular (Mn), maxillary (Mx), and labial (L) segments are denoted by brackets. (B) The mandibular mesoderm expression resolves into two distinct clusters. (C) Faint expression is detected in the maxillary segment. (D) Expression in the maxillary and labial segments becomes restricted to the developing limb buds. The staining in the posterior region is nonspecific. (E and F) *mxp* expression intensifies in the maxillary and labial appendages as they lengthen. (G) Two clusters of cells beneath the surface of the intercalary (Ic) segment begin to express *mxp*. (H) During germband shortening, *mxp* is expressed very strongly in the maxillary and labial appendages and in a segmentally repeated pattern in the CNS.

specific dominant phenotype), were either outcrossed again to generate one-half wild-type and one-half heterozygous embryos, or mated *inter se* to generate one-quarter wild types, one-half heterozygotes, and one-quarter homozygotes. Embryos from both crosses were subjected to *in situ* hybridization with antisense *mxp* riboprobe. Several *mxp* mutant alleles were associated with changes in the *mxp* embryonic expression pattern. In the outcross, mutant heterozygotes were identified by abnormal expression patterns, whereas mutant homozygotes from the self-cross were recognized by patterns not observed in the outcross progeny.

TABLE 2

***mxp* expression in *mxp* mutants**

	Expression domain					
Genotype	An	Man	Max	Lab	Thorax	CNS
Wild type	−	+	+	+	−	+
$mxp^{Dch\text{-}3}$ / $mxp^{Dch\text{-}3}$	−	−	−	+[a]	+	−
$mxp^{Dch\text{-}4}$ / $mxp^{Dch\text{-}4}$	+[b]	−	+[b]	+[b]	+[b]	−
mxp^{Stm} / +	+	+	+	+	−	+
mxp^{StmR1} / mxp^{StmR1}	−	−	−	−	−	−
mxp^{Stb} / +	−	+	+	+	−	+
mxp^{Ng} / mxp^{Ng}	−	+	+	+	−	+
$mxp^{Dch\text{-}1}$ / $mxp^{Dch\text{-}1}$	−	+	+	+	−	+
Apl / Apl^{c}	−	+	+	+	−	+
mxp^{19} / mxp^{19}	−	+	+	+	−	+

[a] It is unclear if the labial segment expression seen represents wild-type expression.
[b] Expression is confined to the distal tips of appendages.
[c] *Apl* is a putative GOF *mxp* allele.

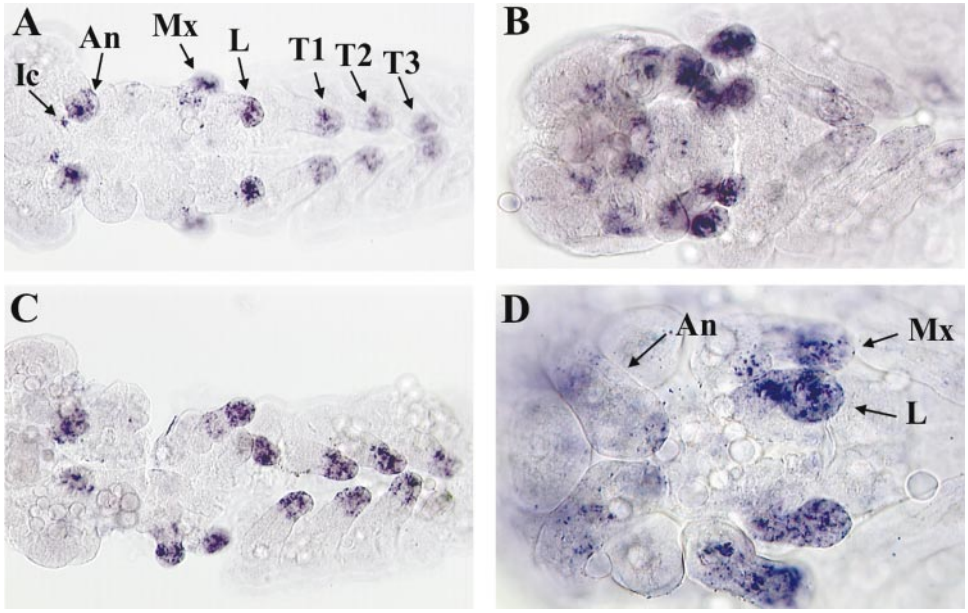

Figure 3.—*mxp* expression in $mxp^{Dch\text{-}4}$ and mxp^{Stm} embryos. All images are ventral views with anterior to the left. (An) Antennal; (Ic) intercalary; (Mx) maxillary; (L) labial; (T1, T2, T3) first, second, and third thoracic segments, respectively. (A) In an $mxp^{Dch\text{-}4}$ heterozygote, faint *mxp* expression is visible in the normal domain. In addition, strong expression is visible in the tips of the antennal, maxillary, labial, and thoracic appendages. (B) During germband retraction, *mxp* expression remains strong in the antennal, maxillary, and labial appendages, but fades in the thoracic appendages. (C) In an $mxp^{Dch\text{-}4}$ homozygote, all normal *mxp* expression is absent (compare to Figure 2G). Expression is seen, however, in the tips of the antennae, maxillary and labial palps, and legs. (D) mxp^{Stm} is associated with ectopic *mxp* expression in the antennae. Expression in heterozygotes and homozygotes is apparently identical.

$mxp^{Dch\text{-}4}$*:* The early *mxp* expression pattern in the gnathal segments of $mxp^{Dch\text{-}4}$ heterozygotes (data not shown) appears similar to that seen in wild-type embryos. After germband extension, ectopic *mxp* expression appears in the tips of the developing antennae and thoracic limbs (Figure 3A). *mxp* expression in the mandibular segment is noticeably reduced relative to wild type. Later, expression in the labial, maxillary, and antennal segments intensifies, but expression in the thoracic appendages fades slightly (Figure 3B). CNS staining is faint relative to wild type (data not shown). Since the homozygotes appear to lack all CNS expression (see below), we interpret this fainter staining in heterozygotes to reflect expression only from the wild-type *mxp* allele.

In $mxp^{Dch\text{-}4}$ homozygotes, *mxp* expression is never detected in the mandibular or intercalary segments (Figure 3C) and is also absent from the CNS (data not shown). In fact, there is no early *mxp* expression in any gnathal segment. However, *mxp* expression in the tips of the maxillary and labial appendages appears coincident with ectopic expression in the tips of the thoracic limbs and antennae. Although the legs and antennae of $mxp^{Dch\text{-}4}$ heterozygous and homozygous larvae appear normal, ectopic expression in the distal portions of thoracic legs corresponds to the transformation of that portion of the adult leg to palp in homozygous adult escapers. The expression of *mxp* in the tips of head, gnathal, and thoracic appendages may represent novel regulation of the *mxp* gene, even within the normal domain (*i.e.*, *mxp* expression restricted to tips of maxillary and labial appendages). The very weak maxillary and labial transformations in $mxp^{Dch\text{-}4}$ homozygotes (see above and Figure 1F) indicate that this tip-specific expression is nearly sufficient for proper gnathal development. Interestingly, it is the distal tips of the palps (where *mxp* is expressed) that are visibly transformed.

$mxp^{Dch\text{-}3}$*:* In $mxp^{Dch\text{-}3}$ heterozygotes, ectopic expression of *mxp* in the first thoracic segment (T1) appears simultaneously with normal expression in the mandibular mesoderm (Figure 4A). As the germband elongates, additional ectopic expression appears at the ventral midline in T2 and T3 (Figure 4C). During development of the thoracic legs, *mxp* expression remains strong in T1, but is greatly reduced in T2 and T3 (Figure 4E). Correspondingly, the larval T1 legs are shortened (see Figure 1H). *mxp* expression in the gnathal segments appears similar to, but fainter than, that in wild type (compare Figures 2 and 4). Likewise, the pattern of *mxp* expression in the CNS (data not shown) is similar to, but fainter than, that in wild-type embryos. As with $mxp^{Dch\text{-}4}/+$, reduced signal in the normal domain apparently reflects expression from the single wild-type allele.

Although $mxp^{Dch\text{-}3}$ homozygotes die before hatching (see above), we could recognize this class among developing embryos by abnormalities of the normal *mxp* expression pattern. The pattern of *mxp* expression in the thoracic segments of $mxp^{Dch\text{-}3}$ homozygotes is similar to that observed in heterozygotes (Figure 4, B and D), but the mandibular and maxillary aspects of *mxp* expression are absent (Figure 4F). There is no evidence of *mxp* expression in the central nervous system. Although *mxp* is expressed in the labial appendages of $mxp^{Dch\text{-}3}$ homozygous embryos, the labial palps of $mxp^{Dch\text{-}3}/mxp^{-}$ indi-

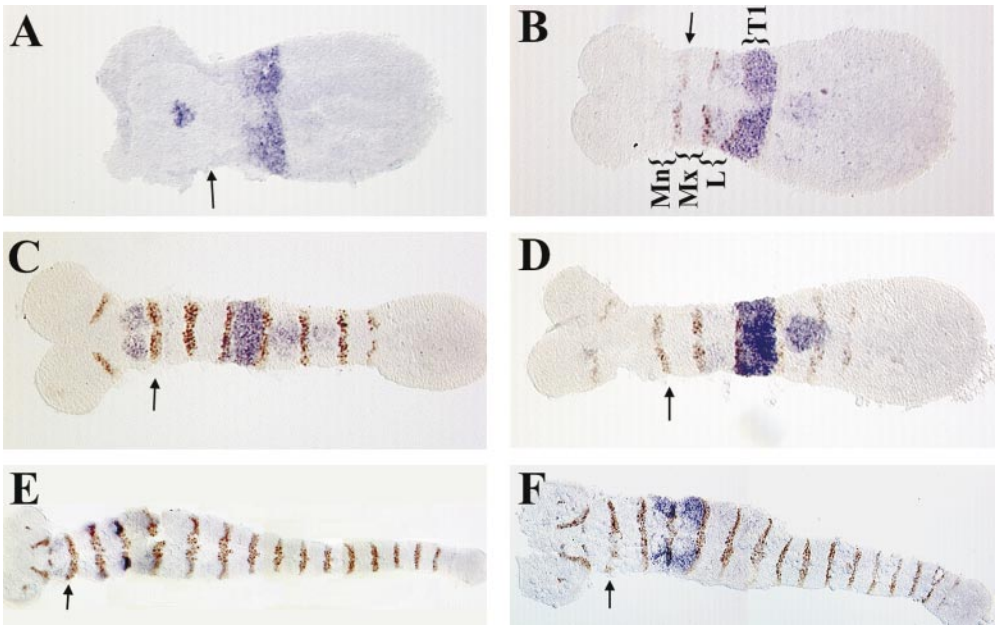

Figure 4.—*mxp* expression in $mxp^{Dch\text{-}3}$ embryos. A, C, and E are $mxp^{Dch\text{-}3}$/+ embryos; B, D, and F are $mxp^{Dch\text{-}3}/mxp^{Dch\text{-}3}$ embryos. All embryos are stained for *mxp* (purple), and all but A are also stained for En (brown). The mandibular (Mn), maxillary (Mx), labial (L), and first thoracic (T1) segments are denoted in B by brackets. An arrow points to the position of the mandibular En stripe in each embryo (position in A is approximate). All embryos are viewed from the ventral side with anterior to the left. (A) In $mxp^{Dch\text{-}3}$ heterozygotes, ectopic *mxp* expression in T1 appears just as expression in the mandibular mesoderm arises. (B) An $mxp^{Dch\text{-}3}$ homozygous embryo of about the same age as A lacks mandibular mesoderm expression. (C) Later in embryogenesis, ectopic *mxp* expression is also seen in T2 and T3 of an $mxp^{Dch\text{-}3}$ heterozygote. (D) Faint expression begins to appear in the labial segment of an $mxp^{Dch\text{-}3}$ homozygote as ectopic expression becomes visible in T2 and T3. (E) Expression in the normal *mxp* domain is fainter in an $mxp^{Dch\text{-}3}$ heterozygote than in wild-type embryos (see Figure 2E), except in the labial segment. (F) In an $mxp^{Dch\text{-}3}$ homozygote, *mxp* is expressed strongly in the labial segment and T1 and faintly in T2 and T3. It is not clear whether the observed labial expression represents wild-type or novel expression.

viduals are partially transformed to leg (see above and Figure 1I).

mxp^{Stm}: Homozygous mxp^{Stm} embryos were obtained from a homozygous stock, and heterozygous embryos were generated by an outcross to wild type. Homozygotes and heterozygotes were indistinguishable and showed ectopic expression in the antennae (Figure 3D). All other aspects of the expression pattern appeared normal. Since the original mxp^{Stm} mutation is homozygous lethal, it is possible that the homozygous mxp^{Stm} stock acquired a wild-type allele of *mxp* while retaining the mutant allele. The normal expression in the wild-type *mxp* domain might reflect the presence of this additional copy.

mxp^{StmR1}: mxp^{StmR1} is a revertant of mxp^{Stm} that lacks the gain-of-function antennal transformation and acts as a typical null *mxp* allele (see above). No ectopic antennal expression is seen in embryos from an mxp^{StmR1}/A^{Es} self-cross, and half of the embryos show no *mxp* expression. A^{Es} homozygotes (one-quarter of the progeny) die early in embryogenesis before *mxp* is expressed. It seems likely that the mxp^{StmR1} lesion eliminates *mxp* expression and that the remaining unstained embryos are mxp^{StmR1} homozygotes.

***mxp* alleles not associated with changes in embryonic *mxp* expression:** A number of *mxp* alleles for which *mxp* expression was assayed showed no changes in expression pattern. The GOF/LOF alleles mxp^{Stb}, mxp^{Ng}, and $mxp^{Dch\text{-}1}$, as well as the putative GOF allele, *Apl*, showed no abnormalities in *mxp* expression in either heterozygotes or homozygotes. The lack of ectopic embryonic expression from these alleles is not particularly surprising since their dominant effects are limited to adult structures. Most of these alleles, however, are also associated with embryonic LOF effects (see above). mxp^{Stb} and $mxp^{Dch\text{-}1}$ cause only partial mouthpart to leg transformations, so levels of *mxp* expression in the normal domain may be only moderately reduced. mxp^{Ng} apparently lacks all normal *mxp* function, but is capable of causing dominant head capsule abnormalities. It is feasible that a nonfunctional protein is produced both in the normal domain and ectopically (in the adult). Such a protein might interfere with the action of endogenous proteins, in a manner analogous to the reported dominant negative effect of nonfunctional Pb on Sex combs reduced (Percival-Smith *et al.* 1997). The null allele mxp^{19}, which is predicted to encode a truncated, nonfunctional Mxp protein (Shippy *et al.* 2000), appears to retain normal *mxp* expression, since virtually all offspring from a self-cross display wild-type expression (data not shown).

DISCUSSION

We have classified available *mxp* alleles by classical genetic methods. Null *mxp* alleles cause larval lethality with strong transformation of maxillary and labial palps to legs, consistent with the hypothesis that an ancestral *pb*-like gene had an embryonic function. Hypomorphic *mxp* alleles cause incomplete transformations of larval

(and sometimes adult) mouthparts to leg. In contrast, the effects of hypomorphic *pb* alleles are visible only in adult flies, where they cause transformation of the distal portion of the labial palps to antennal aristae. The weakest *mxp* phenotype, seen in many null heterozygotes and in some homozygous hypomorphs, is the presence of an abnormally long spike on an otherwise normal maxillary palp. The significance of this spike is unclear, but we have seen similar structures on the maxillary and labial palps of embryos lacking *TcDfd* (S. J. Brown, unpublished results). Since *TcDfd* is not expressed in the labial palps, it is possible that perturbation of normal head development results in an unspecialized sensory structure.

The *mxp* LOF phenotypes are consistent with the wild-type *mxp* expression pattern, since the epidermal sites of embryonic *mxp* expression are the maxillary and labial appendages. Although *mxp* is expressed in both the telopodite and endite of the maxillary appendages, the maxillary endites are unaffected in *mxp* mutants (see Figure 1B). In contrast, the maxillary appendages of embryos homozygous for a null allele of *TcDfd* lack endites, but have relatively normal telopodites (Brown *et al.* 1999). Thus, *mxp* and *TcDfd* apparently function in distinct domains of the embryonic maxillary palps. A strictly additive model based on these data predicts that the maxillary appendages of a Tribolium embryo lacking both *mxp* and *TcDfd* would be transformed toward legs (as in *mxp* nulls) but would lack endites. However, according to the current paradigm, homeotic gene function is necessary in appendages to repress *homothorax* and thus prevent antennal identity. Embryos lacking both *mxp* and *TcDfd* would have no homeotic genes expressed in the maxillary appendages, and thus the maxillary palps might be transformed to antennae. We are currently performing RNAi experiments to test these predictions.

In Drosophila embryos, the maxillary segment has no true appendage, but the maxillary sense organ is believed to be a vestige of the telopodite (Jürgens *et al.* 1986). Interestingly, the maxillary sense organ is present in *Dfd* null mutants (Merrill *et al.* 1987; Regulski *et al.* 1987), suggesting that the *Dfd*-independent development of the maxillary telopodite evolved in a common ancestor to beetles and flies. It seems probable that, in that same common ancestor, a *pb*-like gene specified the identity of the maxillary telopodite. However, all larval structures, including the maxillary sense organ, are apparently normal in *pb* null Drosophila larvae.

It is intriguing that the embryonic expression patterns of *mxp* and Pb are so similar, since *pb* has no apparent function in Drosophila embryos. The similar expression patterns could be interpreted to suggest that the two genes share a common regulatory mechanism. Studies of *pb* regulation (Kapoun and Kaufman 1995) using a *pb* minigene and a *lacZ* reporter gene have revealed the existence of both positive and negative regulatory elements. Region-specific enhancers of *pb* expression are located within its second intron. The region upstream of *pb* is not required for its normal spatial expression pattern. However, removal of this region from a *pb* minigene driven by the *pb* basal promoter results in ectopic expression in the eye-antennal and leg discs. The only observable effect of this ectopic expression is a thickening of the antennal aristae. In contrast, when the *pb* minigene is driven with the Hsp70 promoter (without heat induction) the adult antennae are transformed toward maxillae (Cribbs *et al.* 1995). Similarly, *pb* expression driven by the *decapentaplegic* promoter was shown to transform the adult legs toward a labial identity (Aplin and Kaufman 1997).

GOF alleles of *mxp*, like ectopic expression of *pb* driven by a foreign promoter, transform adult legs and/or antennae toward palps (Beeman *et al.* 1989). Somewhat surprisingly, since most of the GOF effects are limited to adult structures, we have, in some cases, correlated transformations with ectopic embryonic (and presumably adult) *mxp* expression. Of these mutations, only $mxp^{Dch\text{-}3}$ (which produces very strong T1 expression and a corresponding shortening of the embryonic prothoracic legs) is associated with embryonic GOF effects, suggesting that embryonic limbs may be less sensitive to perturbation by ectopic *mxp* expression. The frequent occurrence of *mxp* GOF alleles affecting the antennae and legs suggests that *mxp* expression, like that of *pb*, is normally negatively regulated by upstream antennal and thoracic silencers. Why are GOF mutations, apparently resulting from separation of these elements from the transcription unit, so common for *mxp* when they are never found for *pb*? Since a *pb* minigene lacking the upstream silencer-containing region can, under control of certain promoters, cause transformation of antennae and legs toward palps, the simplest explanation is that the level of *pb* expression driven by the endogenous *pb* promoter is insufficient to produce recognizable transformations. It is also possible that GOF mutations of *pb* are usually embryonic lethal, since heat-shock-induced ubiquitous expression of *pb* during early embryogenesis inhibited germband retraction and head involution (Percival-Smith *et al.* 1997).

It is interesting to note that while some mutations associated with adult GOF effects cause ectopic embryonic *mxp* expression, other mutations with similar adult phenotypes do not. Perhaps independent silencer elements exist for control of embryonic and adult expression. Alternatively, one class of mutants may result from juxtaposition of novel enhancers.

A precise model of *mxp* regulation cannot yet be built. Numerous potential explanations exist for the modified expression patterns observed in *mxp* mutants. Since the molecular lesions associated with these mutations have not yet been characterized, it is possible that complex rearrangements have occurred. Furthermore, it is not clear whether loss of *mxp* expression in portions of the normal domain is caused by introducing upstream silencers or removing endogenous enhancers. Likewise,

ectopic expression might result from either removal of silencers or juxtaposition of novel enhancers. For example, the *mxp* expression pattern in $mxp^{Dch\text{-}3}$ homozygotes is reminiscent of the expression pattern of the *Tribolium Sex combs reduced* ortholog *Cephalothorax* (*Cx*; our unpublished results), raising the possibility that *Cx* regulatory elements have been juxtaposed with the *mxp* transcription unit. In $mxp^{Dch\text{-}4}$ homozygotes, where expression is seen only in the tips of antennal, gnathal, and trunk appendages, the expression pattern may represent a completely novel pattern. Conversely, this pattern could result from removal of silencers, and a reduction in the level of expression in the normal domain. Consistent with the latter scenario, Kapoun and Kaufman (1995) proposed that the region upstream of *pb* contains sequences that upregulate *pb* expression throughout its normal domain.

Our analysis of *mxp* mutant alleles has raised numerous questions about *mxp* regulation. Answering these questions will require several approaches. Identification of molecular lesions associated with particular *mxp* alleles may be useful in broadly defining important regulatory regions. Reporter gene assays (similar to those performed for *pb*) should determine the location of particular types of regulatory elements. With the recent development of a system for transforming Tribolium (Berghammer *et al.* 1999), these experiments are now feasible. Once regulatory regions are identified, sequence comparison between *mxp* and its orthologs might reveal conserved sequences, the functional significance of which can be tested by targeted mutagenesis.

We thank Marcé Lorenzen, Kathryn Hummels, and Sandra Koo for technical assistance, and Mark DeCamillis for allowing the Lucifer Revertant 1 mutation to be used prior to publication. This is contribution number 00-286J from the Kansas Agricultural Experiment Station. This work was supported by National Science Foundation grant MCB-9630179 and National Institutes of Health grant HD29594 to R.E.D., R.W.B., and S.J.B. T.D.S. was supported by a Virology and Tumor Biology predoctoral training grant from the National Institutes of Health.

LITERATURE CITED

Aplin, A. C., and T. C. Kaufman, 1997 Homeotic transformation of legs to mouthparts by proboscipedia expression in Drosophila imaginal discs. Mech. Dev. **62:** 51–60.

Averof, M., and N. H. Patel, 1997 Crustacean appendage evolution associated with changes in Hox gene expression. Nature **388:** 682–686.

Beeman, R. W., T. R. Johnson and S. M. Nanis, 1986 Chromosome rearrangements in *Tribolium castaneum*. J. Hered. **77:** 451–456.

Beeman, R. W., J. J. Stuart, M. S. Haas and R. E. Denell, 1989 Genetic analysis of the homeotic gene complex (HOM-C) in the beetle *Tribolium castaneum*. Dev. Biol. **133:** 196–209.

Berghammer, A. J., M. Klingler and E. A. Wimmer, 1999 A universal marker for transgenic insects. Nature **402:** 370–371.

Bridges, C., and T. Dobzhansky, 1933 The mutant '*proboscipedia*' in *Drosophila melanogaster*—A case of hereditary homöosis. Roux Arch. Dev. Biol. **127:** 575–590.

Brown, S. J., N. H. Patel and R. E. Denell, 1994a Embryonic expression of the single *Tribolium engrailed* homolog. Dev. Genet. **15:** 7–18.

Brown, S. J., R. B. Hilgenfeld and R. E. Denell, 1994b The beetle *Tribolium castaneum* has a *fushi tarazu* homolog expressed in stripes during segmentation. Proc. Natl. Acad. Sci. USA **91:** 12922–12926.

Brown, S. J., J. P. Mahaffey, M. L. Lorenzen, R. E. Denell and J. W. Mahaffey, 1999 Using RNAi to investigate orthologous homeotic gene function during development of distantly related insects. Evol. Dev. **1:** 11–15.

Cribbs, D. L., A. M. Pattatucci, M. A. Pultz and T. C. Kaufman, 1992 Ectopic expression of the Drosophila homeotic gene *proboscipedia* under *Antennapedia* P1 control causes dominant thoracic defects. Genetics **132:** 699–711.

Cribbs, D. L., C. Benassayag, F. M. Randazzo and T. C. Kaufman, 1995 Levels of homeotic protein function can determine developmental identity: evidence from low-level expression of the *Drosophila* homeotic gene *proboscipedia* under Hsp70 control. EMBO J. **14:** 767–778.

Denell, R. E., S. J. Brown and R. W. Beeman, 1996 Evolution of the organization and function of insect homeotic complexes. Semin. Cell Dev. Biol. **7:** 527–538.

Diederich, R. J., A. M. Pattatucci and T. C. Kaufman, 1991 Developmental and evolutionary implications of *labial*, *Deformed* and *engrailed* expression in the Drosophila head. Development **113:** 273–281.

Finnerty, J. R., 1998 Homeoboxes in sea anemones and other nonbilaterian animals: implications for the evolution of the Hox cluster and the zootype. Curr. Top. Dev. Biol. **40:** 211–254.

Hoy, M. A., 1966 Section on new mutants. Tribolium Info. Bull. **9:** 85.

Jürgens, G., R. Lehmann, M. Schardin and C. Nüsslein-Volhard, 1986 Segmental organisation of the head in the embryo of *Drosophila melanogaster*. Roux Arch. Dev. Biol. **195:** 359–377.

Kapoun, A. M., and T. C. Kaufman, 1995 A functional analysis of 5′, intronic and promoter regions of the homeotic gene *proboscipedia* in *Drosophila melanogaster*. Development **121:** 2127–2141.

Kaufman, T. C., 1978 Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: isolation and characterization of four new alleles of the *proboscipedia* (*pb*) locus. Genetics **90:** 579–596.

Kaufman, T. C., M. A. Seeger and G. Olson, 1990 Molecular and genetic organization of the Antennapedia gene complex of *Drosophila melanogaster*. Adv. Genet. **27:** 309–362.

Kennerdell, J. R., and R. W. Carthew, 1998 Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. Cell **95:** 1017–1026.

Lewis, E., 1978 A gene complex controlling segmentation in Drosophila. Nature **276:** 141–152.

Merrill, V. K. L., F. R. Turner and T. C. Kaufman, 1987 A genetic and developmental analysis of mutations in the *Deformed* locus in *Drosophila melanogaster*. Dev. Biol. **122:** 379–395.

Misquitta, L., and B. M. Paterson, 1999 Targeted disruption of gene function in drosophila by RNA interference (RNA-i): a role for nautilus in embryonic somatic muscle formation. Proc. Natl. Acad. Sci. USA **96:** 1451–1456.

Patel, N. H., T. B. Kornberg and C. S. Goodman, 1989 Expression of *engrailed* during segmentation in grasshopper and crayfish. Development **107:** 201–212.

Percival-Smith, A., J. Weber, E. Gilfoyle and P. Wilson, 1997 Genetic characterization of the role of the two HOX proteins, Proboscipedia and Sex Combs Reduced, in determination of adult antennal, tarsal, maxillary palp and proboscis identities in *Drosophila melanogaster*. Development **124:** 5049–5062.

Pultz, M. A., R. J. Diederich, D. L. Cribbs and T. C. Kaufman, 1988 The *proboscipedia* locus of the Antennapedia complex: a molecular and genetic analysis. Genes Dev. **2:** 901–920.

Regulski, M., N. McGinnis, R. Chadwick and W. McGinnis, 1987 Developmental and molecular analysis of *Deformed*, a homeotic gene controlling *Drosophila* head development. EMBO J. **6:** 767–777.

Rogers, B. T., M. D. Peterson and T. C. Kaufman, 1997 Evolution of the insect body plan as revealed by the Sex combs reduced expression pattern. Development **124:** 149–157.

Ruddle, F. H., J. L. Bartels, K. L. Bentley, C. Kappen, M. T. Murtha *et al.*, 1994 Evolution of *Hox* genes. Annu. Rev. Genet. **28:** 423–442.

Shippy, T. D., S. J. Brown and R. E. Denell, 2000 *maxillopedia* is the *Tribolium* ortholog of *proboscipedia*. Evol. Dev. **2:** 145–151.

Sokoloff, A., 1982 Section on new mutants. Tribolium Info. Bull. **22:** 79.

Communicating editor: T. C. Kaufman